

Moderate stripping solution
 200 mM Tris-Cl, pH 7.0
 0.1× SSC (APPENDIX 2)
 0.1% (w/v) SDS

Nucleotide mix

2.5 mM ATP
 2.5 mM CTP
 2.5 mM GTP
 20 mM Tris-Cl, pH 7.5
 Store at -20°C

COMMENTARY

Background Information

Hybridization between complementary nucleotides was implicit in the Watson-Crick model for DNA structure and was initially exploited, via renaturation kinetics, as a means of studying genome complexity. In these applications, the two hybridizing molecules were both in solution—an approach that is still utilized in “modern” techniques such as case protection transcript mapping (UNITS 6.2) and oligonucleotide-directed mutagenesis (Chapter 8). The innovative idea of immobilizing one hybridizing molecule on a solid support was first proposed by Denhardt (1966) and led to methods for identification of specific sequences in genomic DNA (dot blotting; UNIT 6.3) and recombinant clones (UNITS 6.3 & 6.4). A second dimension was subsequently introduced by Southern (1975), who showed how molecules contained in an electrophoretic gel could be transferred to a membrane (UNIT 6.4), enabling genetic information relating to individual restriction fragments to be obtained by hybridization analysis.

Since the pioneering work of Denhardt and Southern, advances in membrane hybridization have been technical rather than conceptual. Reviewed by Dyson (1991), the detailed protocols have become more sophisticated, not because of advances in understanding the factors that influence hybrid stability and hybridization rate.

Hybrid stability is expressed as the melting temperature or T_m , which is the temperature at which the probe dissociates from the target DNA. For DNA-DNA hybrids, the T_m can be estimated from the equation of Meinkoth and Melnick (1984):

$$T_m = 69.3^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\%form) - 500/L$$

for DNA-DNA hybrids from the equation of Dyson and Davidson (1977):

$$T_m = 69.3^\circ\text{C} + 18.5 (\log M) + 0.58 (\%GC) - 11.8 (\%GC)^2 - 0.56 (\%form) - 820/L$$

where M is the molarity of monovalent cations, and L is the percentage of guanosine and cyto-

sine nucleotides in the DNA, %form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The practical considerations that arise from these two equations are summarized Table 2.10.2A.

The second important consideration in hybridization analysis is the rate at which the hybrid is formed. Hybrid formation cannot occur until complementary regions of the two molecules become aligned, which occurs purely by chance; however, once a short nucleating region of the duplex has formed, the remaining sequences base-pair relatively rapidly. The rate at which the probe “finds” the target, which is influenced by a number of factors (Table 2.10.2B), is therefore the limiting step in hybrid formation (Britten and Davidson, 1985). However, in practical terms hybridization rate is less important than hybrid stability, as in most protocols hybridization is allowed to proceed for so long that factors influencing rate become immaterial.

Critical Parameters

To be successful, a hybridization experiment must meet two criteria:

(1) **Sensitivity.** Sufficient probe DNA must anneal to the target to produce a detectable signal after autoradiography.

(2) **Specificity.** After the last wash, the probe must be attached only to the desired target sequence (or, with heterologous probing, family of sequences).

Parameters influencing these two criteria are considered in turn, followed by other miscellaneous factors that affect hybridization.

Factors influencing sensitivity

The sensitivity of hybridization analysis is determined by how many labeled probe molecules attach to the target DNA. The greater the number of labeled probe molecules that anneal, the greater the intensity of the hybridization signal seen after autoradiography.

Probe specific activity. Of the various factors that influence sensitivity, the one that most frequently causes problems is the specific ac-

Preparation and Analysis of DNA

2.10.8

Supplement 29

EXHIBIT A

e.g. expression vectors, the necessary common attribute is the ORF (SEQ ID NO: 2).

Weighing all factors including (1) that the full length ORF (SEQ ID NO: 2) is disclosed and (2) that any substantial variability within the genus arises due to addition of elements that are not part of the inventor's particular contribution, taken in view of the level of knowledge and skill in the art, one skilled in the art would recognize from the disclosure that the applicant was in possession of the genus of DNAs that comprise SEQ ID NO: 2.

Conclusion: The written description requirement is satisfied.

Example 9: Hybridization

Specification: The specification discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification includes an example wherein the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) for the isolation of nucleic acids that encode proteins that bind to dopamine receptor and stimulate adenylate cyclase activity. The hybridizing nucleic acids were not sequenced. They were expressed and several were shown to encode proteins that bind to a dopamine receptor and stimulate adenylate cyclase activity. These sequences may or may not be the same as SEQ ID NO: 1.

Claim:

An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1,

wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Analysis:

A review of the full content of the specification indicates that the essential feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing.

The claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with a specific activity.

The search of the prior art indicates that SEQ ID NO: 1 is novel and unobvious.

There is a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus.

There is actual reduction to practice of the disclosed species.

Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of

skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Conclusion: The claimed invention is adequately described.

gi|2251107:452-1837 Pyrococcus
SEQ ID NO:125

10 20 30 40 50 60
ATG---AAGAAGTTTGTG---CCCTGCTCATAACCATGTTTTCGTAGTGAGCATGG
gtggtgcacatgaagtt-gaagtaccttgc-cttag---ttttgttgggtgtg-gcttgcg

70 80 90 100 110 120
CT---GCCGTGACACAGC---CAGCTAGCGCCGCAAGTATTCGGAAGTCCGAAGAGGCGGC
ataggectactctctgactccagtggtgtgcaagtaactccgaactcgaagagggcggt

130 140 150 160 170 180
GTTATAATGCAGGCTTCTACTGGGACGTTCCAGCGGAGGAATCTGGTGGGATACAAATC
gttataatgcaggccttctactgggatgttcccggagggggaatctggtgggacaccata

190 200 210 220 230 240
AGA-AGCAAGATACCGGAGTGGTACGAGGCTGGAATCTCCGCAATCTGGATTCCGCCAGC
agacagaaa-atcccgagtggtacgaagctggaatctcggcgatatggattctctccagc

250 260 270 280 290 300
CAGCAAGGCATGGGAGGAGCTTATTCAATGGGCTACGACCCATACGACTTCTTGCACCT
tagcaaaagggatgggaggtgttatttcacgggctacgatccctacgatcttctttgacct

310 320 330 340 350 360
CGCGGAGTACAAACCAGAAGGGAACAGTTGAAACTCGCTTGGCTCAAAGCAGGAGCTTAT
cggcgagtactatcagaagggaacagttggaagcgcttcggetcaaaaggaggaactggt

370 380 390 400 410 420
CAACATGATAAACACCGCCCATGCTTACGGCATAAAGGTCATAGCTGATATCGTCATAAA
gaacatgataaacaccgcacactcctatggcataaaggtagataggacatagtcataaa

430 440 450 460 470 480
CCACCGCGCGGGCGGAGACCTCGAGTGGAAACCCGTTCTGGGGACTACACTGGACCGA
ccaccgcgcgggtggagaccttgagtggaaaccccttgtaaacactatacttggacaga

490 500 510 520 530 540
CTTCTCGAAGGTGGCTCGGGCAATATACCGCAACTACCTCGACTTCCACCCCAACGA
cttctcgaaggtcgctcggtaataacaggccaactaccttgacttccacccaacga

550 560 570 580 590 600
GCTCAAGTGCTGTGACGAGGGACATTTGAGGTTTCCAGACATAGCCACGAGAAGGA
ggtcaagtgtgctgagtgagggtacatttggtagcttccggacatcgccacagagaag-a

610 620 630 640 650 660
G-TGGGACGAGCACTGGCTCTGGGCCAGCCACGAGAGCTACGCCCGCTACTCAGGAGCA
gctgggatcagtaactggtctctgggcaagcaatgagagctacgcccgatactctcggagca

670 680 690 700 710 720
TCGGCGTTGATGCCGTCGGCTTTGACTACGTAAGGCTACCGAGCGTGGGTCTGTCAGG
tagggatcgatgcattggcggtttcgactacgtcaagggtacggagcggtgggtgtt-aatg

730 740 750 760 770 780
ACTGGCTCAACTGGTGGGGCGGCTGGGCCGTGGGAGTACTGGACACGACGTTGATG
actggctcagctggtggggaggctgggcccgttggagagtactgggacacgaacgttgatg

790 800 810 820 830 840
CACTCCTCAATGGGCATACTCCAGCGGCACCAAGGTCTTCGACTTCCCGCTCTACTACA
cactcctcaactgggcatacagacgggtgccaaggctcttgacttcccgcctctactaca

EXHIBIT C

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....850.....860.....870.....880.....890.....900
AAATGGACGAGGCCTTTGACAAACACCAACATCCCGGCCTTGGTTTCATGCCCTCCAGAACG
agatggacgaagcctttgacaacaccaacatccccgctttggtttacgcctccagaacg

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....910.....920.....930.....940.....950.....960
GGGGAACCGTCGTCTCTCGCGACCCGTTCAAGGCCGTAACCTTAGTAAACACGACA
gaggaacagtcgtttcccgcgatccccttcaaggcagtaactttcgttgccaaccacgata

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....970.....980.....990.....1000.....1010.....1020
CCGATATAATCTGGAACAAGTACCGTGCTTATGCTTTCATCCTACCTACGAGGGCCAGC
cagatataatctggaacaagtatcccggcttatggttccatccctacctaagggaacg

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1030.....1040.....1050.....1060.....1070.....1080
CCGTCATATTTACCGCGACTACGAGGAGTGGCTCAACAAGGCAAGCTTAACAACCTAA
ctgttatattttaccgcgactacgaggagtggttcaacaaggataagcttaacaacctta

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1090.....1100.....1110.....1120.....1130.....1140
TCTGGATACACGACACCTCGCGGGTGAAGCACAGCATACTCTACTACGACAGCGACG
tctggatacacgagcaccttcgagggaagtacaaagatccctactactacgataacgag

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1150.....1160.....1170.....1180.....1190.....1200
AGCTCATCTTCGTGAGGAACGGCCACTCCAAGAGCCCGGCACTCATAACCTACATCAACC
agctaatattctatgagggaaggctacgggagcaaggccgggctcataacctacataaacc

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1210.....1220.....1230.....1240.....1250.....1260
TCGGCTCTAGCAAGGTCGAAAGGTGGGTGTACGTGC-CAAGTTCCGGGGCGGTGC
tcggaaacga-ctgggcccagagcgtgggtgaacgtcgggtcaagtttgcgggtacaca

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1270.....1280.....1290.....1300.....1310.....1320
ATCCACGACTACACCGGCAACCTCGGAGGCTGGGTAGACAAGTACGTCCAGT-CGACCGG
atccatgaatacacaggcaatctcgggtggctgggttgacaagtggttcagtagcat-gg

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1330.....1340.....1350.....1360.....1370.....1380
CTGGGTCTATCTCGAAG-CTCCAGCTTACGAACCCGCCACCGGGCACTACGGCTACACCG
atgggttaaat-gaaggcacctctcatgacccagccaacggataattacggctactcag

gi|2251107:452-1837 *Pyrococcus*
SEQ ID NO:125
.....1390.....1400.....
TCTGGAGCTACTGCGGC-GTGGATGA
tctggagctac-gcaggcgtcggatga

SEQ ID NO:126
gi|2251108|dbj|BAA21130.1| alp

10 20 30 40 50 60
-----VHMKLKYLALVLL-----AVASIGLL--STPVGA--AAKYSELEEGGVIMQA
MKRF-V-----A--LLITMFFVVSMAVA-----QPAS-----AAKYSELEEGGVIMQA

70 80 90 100 110 120
FYWDVF--GGGIWWDTIQ--KIPWYD--AGISAIWIPPASKGMGG--YSMGYDPYDFDLG
FYWDVPAGG--IWWDTIR--SKIPEWY--EAGISAIWIPPASKGMGG--AYSMGYDPYDFDLG

130 140 150 160 170 180
EYY--QKGTVETREFGSK--EELV--NMINTAHS--YGIKVIADIVINHRAGGDLEWNPFFVNN--
EY--NQKGTVETREFGSK--E--L--INMINTAH--AYGIKVIADIVINHRAGGDLEWNPFFV--GD

190 200 210 220 230 240
YTWTDFSKVASGKYTANYLDFHPNEVKCCDEGTFC--FPDIAHEKS--WDQY--WLWASN--E
YTWTDFSKVASGKYTANYLDFHPNEVKCCDEGTFC--GFPDIAHEK--EWDQ--HWLWAS--DE

250 260 270 280 290 300
SYAAYLRSIGI--DAWRFDYVKGYGAWV--DWT--SWWGGWAVGEYWDTNVDALLNWAYDS
SYAAYLRSIGI--VDAWRFDYVKGYGAWV--KDWL--WWGGWAVGEYWDTNVDALLNWAY--S

310 320 330 340 350 360
--GAKVFDFPLYKMDFAFDNTNIPALVY--ALQGGTVVSRDFFKAVTFVANHDTDIWNK
SGAKVFDFPLYKMDFAFDNTNIPALV--DALQGGTVVSRDFFKAVTFVANHDTDIWNK

370 380 390 400 410 420
YPAYAFILTYEGQPVIFYRDYEEWLNKDKLNNLIWIHE--HLAGGSTKIL--YYDN--DEL
YPAYAFILTYEGQPVIFYRDYEEWLNKDKLNNLIWIH--DHLAGGST--SIVYYD--SDEL

430 440 450 460 470 480
IFMREGY--G--SK--PGLITYINLNDWAE--RWVNVGS--KFAGYT--THEYTG
IF-----VRNGDSKRPGLITYINL-----SSKVGWV--TVPKFAG--ACIHEYTG

490 500 510 520 530
LGGWVDRWVQYD-----GWVK--LTAP--PH--DPANGY--YGYS--VWSYA--GVG*
LGGWVD-----KYVESSGWV--YL--EAP--AYDPA--SGQYGY--TVWSY--CGVG--

EXHIBIT D

Atty Docket No.: 56446-20061.00 /09010-108001/DIVER1530-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Walter Callen et al. Art Unit : 1652
Serial No. : 10/081,872 Examiner : Rebecca E. Prouty, Ph.D.
Filed : February 21, 2002
Title: Enzymes Having Alpha Amylase Activity and Methods of Use Thereof

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume was provided with my previous declaration submitted in connection with this application.

2. It was considered routine by one skilled in the art to make multiple substitutions or multiple modifications in a nucleic acid sequence, such as a polypeptide coding sequence, and screen for variant enzymes, such as amylases, encoded by the modified nucleic acids. The state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for screening enzymes for amylase activity was very high. It would not have been necessary for the skilled artisan to understand which regions of the amylases encoded by nucleic acids used in the claimed methods could be modified to gain a function or activity, or, modified without loss of a function or activity, for example, amylase activity. It would not have been necessary for the skilled artisan to understand which specific regions or structural elements of the exemplary sequences were needed to have amylase function or activity (e.g., amylase activity) to routinely generate the genus of amylase-encoding nucleic acids used in the claimed methods. Methods for making and screening sequence modifications and enzyme fragments were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate a genus of amylase-encoding sequences without need of knowing which

sd-214331

Applicant : Walter Callen et al.
Serial No. : 10/081,872
Filed : February 21, 2002
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108001/DIVER1530-5

specific regions or structural elements of a sequence or structure affected function or activity (e.g., amylase activity). Methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with high through-put enzyme (amylase) screening made methods that required previous knowledge of structural elements necessary for enzymatic activity obsolete and unnecessary. Methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with high through-put enzyme (amylase) screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. At the time of the invention, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and enzyme (amylase) screening protocols were well known in the art. The specification sets forth exemplary amylase screening assays to determine if a polypeptide is within the scope of the genus used in the claimed methods (see, e.g., Examples 1-2 and 5-10, of the specification). Using methods known in the art at the time of the invention it would not have been necessary to understand which specific regions of amylase structure needed to be modified to generate a genus of nucleic acids or polypeptides for practicing the claimed methods without undue experimentation. The specification presented to the skilled artisan a rational and predictable scheme for making the genus of amylases and amylase-encoding sequences, including a rational and predictable scheme for modifying the exemplary SEQ ID NO:125 with an expectation of obtaining an enzymatically active genus of amylase-encoding nucleic acids or amylase enzymes, including amylases. The specification provided sufficient guidance to one of ordinary skill in the art to make and use the genus of amylase-encoding nucleic acids to practice the methods of the invention.

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Applicant : Walter Callen et al.
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Atty Docket No.: 56446-20061.00 /09010-
108001/DIVER1530-5

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: _____

Jay Short

sd-214331

CURRICULUM VITAE**NAME:** Jay M. Short, Ph.D.**EDUCATION:**

1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

RESEARCH & PROFESSIONAL EXPERIENCE:

<u>1999 - present</u>	CEO, President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
<u>1998 - present</u>	President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
1997 - 1998	Executive Vice President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
1994 - 1997	Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
<u>1990 - 1994</u>	President Stratacyte, Inc. La Jolla, California
<u>1992 - 1994</u>	Vice President R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	Vice President R&D (Research) and Biological Operations Stratagene Cloning Systems
1988 - 1989	Senior Staff Scientist Research and Development Stratagene Cloning Systems

1985 - 1988

Staff Scientist
Research and Development
Stratagene Cloning Systems

1981 - 1985

Ph.D. Research
Case Western Reserve University
Dr. Richard W. Hanson's Laboratory,
Identification and characterization of the promoter for
P-enolpyruvate carboxykinase.
First Identification of a cAMP regulatory domain.

1980 - 1981

Graduate Student Research
Case Western Reserve University
Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of *Trichoderma viride*.

TEACHING EXPERIENCE:

Thesis Advisor (1988-1993), University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees
Lecturer (1992), Committee for Advanced Scientific Education, Center for Drug
Evaluation and Research, FDA.
Faculty (1989), Transgenic Mouse Model and Its Application in Assessing
In Vivo Mutagenesis, Genetic Toxicology Workshop (3rd Annual).
Microbiological Associates Inc. Bethesda, MD.
Faculty (1987), DNA Cloning and Expression. Physiology Society Workshop. San Diego, CA.
Teaching Assist., (1981-1985). Molecular and Cellular Biology. Case Western
Reserve University.
Teaching Assist., (1981). Physiological Chemistry. Kent State Univ., Kent, OH.
Teaching Assist., (1978-1980). Quantitative Analysis. Taylor University.

AWARDS, PROFESSIONAL MEMBERSHIPS, ACCOMPLISHMENTS, AND ACTIVITIES:

Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya (2002-2004)
Science & Technology Committee, *BIOCOM San Diego*
Advisory Board, IngleWood Ventures
Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category
Advisory Board, *Chemical & Engineering News*
Board of Advisors and Founding Member, Division of Biological Sciences, *UCSD*
Board Director, *BIOCOM San Diego*
Chairman of the Board, Innovase
Board Director, Zymetrics
Board Director, Innovase
Director at Large, *YPO (Young Presidents' Organization) San Diego*.
2001 T-Sector Life Science Innovator Award.
2001 Deloitte and Touche's Orange County / San Diego 2001 Technology "Fast 50".
San Diego Entrepreneur of the Year 2001.
YPO (Young Presidents' Organization) San Diego.
YPO (Young Presidents' Organization) International.
Finalist for San Diego Entrepreneur of the Year in 2000.
Largest Biotechnology IPO raising over \$200MM.
Founding management member of Diversa Corporation.
Panel for Chemical Science & Technology for NIST, appointed by the National Research Council (1997-2000).
Chairman (1993), Discussion Group, Society of Toxicology Conference.
U.S. Committee Member for Evaluation of Biotechnology Research in Spain.
Editor, Mutation Research.

UCSD Connect Program (1991) 1st Place Award for Innovation and Entrepreneurship in Biotechnology (over 50 competing biotech companies).
 UCSD Connect Program (1990) 1st Place Award for Innovation and Entrepreneurship in Biotechnology.
 Consultant for European Economic Community on Transgenic Toxicology Testing (91-94).
 The New York Academy of Sciences.
 Reviewer for *Proceedings of the National Academy of Sciences, Genetic Analysis Techniques, Analytical Biochemistry, & Nucleic Acids Research*.
 American Association for the Advancement of Science.
 American Chemical Society.
 American Society of Biochemistry and Molecular Biology.
 American Society of Microbiology.
 Environmental Mutagenesis Society.
 Society for Industrial Microbiology
 Society of Toxicology.
 Japanese Environmental Mutagen Society.
 Who's Who Registry of Business Leaders (1994-1995)
 American Men and Women of Science (1995)
 NIEHS Peer Review Committee.
 SBIR Study Section.
 SBIR Annual Report (1993) Program Success Profile (Top 8 of 800 Companies).
 Stratagene (1990) Innovation Award - Lambda ZAP[®] vector.
 Stratagene (1990) Service Award
 Stratagene (1991) Innovation Award - Big Blue[®] Transgenic Testing System.
 Stratagene (1992) Most Innovative Award - Managers/Supervisors.
 Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official.
 Award from the University of Victoria for Contributions to the Development of Short-term Transgenic Mutation Assays.
 Nominated as Council Member for the U.S. Environmental Mutagen Society.
 Board Director, *Stressgen (TSE), Victoria, BC, Canada*
 Board Director & Treasurer, *Stressgen Therapeutics, Victoria, BC, Canada*
 Board Director & Secretary, *Stressgen Therapeutics, Victoria, BC, Canada*
 Board Director, *Diversa, La Jolla, CA*
 Board Director, *Invitrogen, Carlsbad, CA*
 Consultant, *Stratagene Cloning Systems, La Jolla, CA*
 Consultant, *Micro Product Systems, Lynn, IN*
 Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on *The Human Genome Project and Patenting DNA Sequences*.

MEDIA:

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Directed Evolution of Thermophilic Enzymes (1996).
Environmental Biopanning (1996).
Combinatorial Enzyme Development (1996).
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High Throughput Screening for Novel Enzymes (1997).
"Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, etc.) (1997).
Production of Enzymes Having Desired Activities By Mutagenesis (1999).
Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1999).
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Enzyme Kits and Libraries (1999).
Screening for Novel Bioactivities (2000).
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Saturation Mutagenesis in Directed Evolution (2001).
High Throughput Screening for Novel Enzymes (2001).

Recombinant Bacterial Phytases and Uses Thereof (2001).
 Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid (2001).
 End Selection in Directed Evolution (2001)
 Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same (2001)
 Directed Evolution of Thermophilic Enzymes (2002)
 Method for Screening for Enzyme Activity (2002)
 Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
 End Selection in Directed Evolution (2002)
 Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
 Screening for Novel Bioactivities (2002)
 Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process (2002)
 Production and Use of Normalized DNA Libraries (2002)
 Sequence Based Screening (2002)
 Non-Stochastic Generation of Genetic Vaccines (2002)
 Over 100 Additional Pending Patent Applications Worldwide.

GRANTS AND CONTRACTS:

- *Phase I Small Business Contract #N43-Am-62282. 1985 - 1986. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
- *Phase II Small Business Contract #N43-Am-62282. 1988 - 1990. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
- *Phase I Small Business Grant 2R43ES04484-02. 1986 - 1987. P.I.
Identification of Genetic Lesions Leading to Mutations.
- *Phase II Small Business Grant 2R43ES04484-02. 1989 - 1992. P.I.
Identification of Genetic Lesions Leading to Mutations.
- *1R01-ES04728-01A1. 1989 - 1992. (NIEHS) P.I.
Animal Model for Identification of Genetic Lesions.
- *Phase I Small Business Grant #R43GM42291-01. 1989. P.I.
Switch Mechanism for Gene Expression in Transgenics.
- *RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.
Development of Mutagenesis Assays Using Transgenic Mice.
- *Phase II Small Business Grant #2R44GM42291-02. 1990-1992. (DRG/NIH) P.I.
Switch Mechanism for Gene Expression in Transgenics.
- *Phase I Small Business Grant #1R43GM46585-01. 1991. (DRG/NIH) P.I.
Generation of a Peptide Screening System Through the Development of Combinatorial-splicing "Polycos" Vectors.
- *Phase I Small Business Grant #1R43CA57066-01. 1992. (NCI) P.I.
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens.
- *Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.
Analysis of the Immunoglobulin Hypermutator Mechanism.
- *Phase I Small Business Grant #1R43ES06146-01. 1992. (NIEHS) P.I.
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing.
- *Phase II Small Business Grant #2R44GM46585-02. 1992-1994. (NIGMS) P.I.
Peptide Screening Utilizing Combinatorial Polycos Vector.
- *Phase I Small Business Grant #1R43RR08667-01. 1992-1993. (DRG/NIH) Co-I.
A One-step PCR Cloning System Based on FLP Recombination.
- *Phase II Small Business Grant #2R44CA57066-02. 1993-1995. (NCI) P.I.
Transgenic Rats: Transgenic Rat Model for Mutagenicity Testing.
- *Phase I Small Business Grant. 1993-1994. (NIH) Co-I.
Transgenic Fish Model for Mutagenicity Testing.
- *Phase II Small Business Grant (1994-1996). (NIH) P.I.
"Polycos" Shuttle Vectors for Mutagenicity testing.
- *Phase I Small Business Grant. 1994. (NIH) Co-I.
Vector System for Studying Protein-Protein Interactions.

- *CRADA with LLNL. 1994. (NIH) Co-I.
Mouse and Rat Painting Probes.
- *CRADA with FDA. 1994. (NIH) Co-I.
Tamoxifen Testing in F-344 Rats.
- *CRADA with NASA. 1994. (NIH) Co-I.
Radiation Damage in the Microgravity Environment.

ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.*, 80:3656-3660.
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